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Gestational and Lactational Exposition to Aqueous Leaf Extract of *Jatropha tanjorensis* Impairs Neuro development and Cognitive Function in Juvenile Rats of Wistar Strain

Imoh Emmanuel Ukoh[1]*

[1]Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Federal University Otuoke, Otuoke, Bayelsa State, Nigeria

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***Corresponding author:** Imoh Emmanuel Ukoh
Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Federal University Otuoke, Otuoke, Bayelsa State, Nigeria

Abstract

Expectant and lactating mothers resort to medicinal plants for it claimed nutritional values, such practices effects on offspring neurodevelopment and cognitive functions are unclear. Hence, this study assessed cognitive function and hippocampus in juvenile rat exposed to aqueous leaf extract of *Jatropha tanjorensis* (ALEJT) in-utero and postnatal life. Thirty litters were randomly assigned 3 groups (n=10) following dams intake. Control: 20ml/kg of distilled water; In-utero and In-utero+postnatal days 15-21 ALEJT 500mg/kg oral exposure respectively. Cognitive assay after weaning: Novel object recognition task (NORT); Morris water maze (MWM) and T maze; Histological studies: hematoxylin and eosin staining. NORT short term memory; exposed groups index of habituation increased against control. Index of discrimination differed not. Long term memory: Group 3 index of habituation increased ($p<0.05$) against control, and was positive against other Groups. Exposed groups index of discrimination was negative against control. Group 2 ($p<0.05$) reduced against control. During MWM acquisition training, day 3 swim latency (SL) was shorter in Group 3 ($p< 0.01$) against control. During reversal training, day 1 SL was shorter in Group 3 against control ($p<0.05$) and Group 2 ($p<0.01$); shorter on day 2 ($p<0.05$) against Group 2 and on day 3 ($p<0.01$) against control. During probe trial Group 3 spent time in Northwest quadrant against the retention quadrant (Southwest). Group 3 SL during visible platform was shorter ($P<0.01$) against control. Percentage alternation differed not. Exposed rats hippocampus showed altered pyramidal cells, with reactive astrocytes present. ALEJT impairs cognitive function with potential injuries to hippocampus of exposed rats.

Keywords: Jatropha tanjorensis, neurodevelopment, Gestation, Postpartum, Hippocampus, cognition.

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INTRODUCTION

Brain is a metabolically active organ, its growth occurs early in life and any interruptions or changes in it developing stage, either anatomically or physiologically, can have a detrimental effect on the developing fetus (Atallah and El-Gaber, 2022) such as improper neural development, which leads to behavioral anomalies later in life (Atallah and El-Gaber, 2022). Anatomically, the hippocampus is a vital area for memory and learning development (Xiong *et al*., 2014), and is liable for numerous cognitive functions in various living organisms (Alves-Oliveira *et al*., 2020;). The hippocampus is essential in the consolidation of information from short-term memory (STM) to long-term memory (LTM), and in spatial memory that enables navigation (Amaral and Lavenex, 2006). Changes in maternal metabolism caused by toxicity interfere with offspring growth to the point that cognitive development of the rat offspring can be compromised (de Campos *et al.,* 2014**),** and brain injury during early development results in continuous decrease in hippocampal volumes (Semple *et al.,* 2013).

A careful attention and concern is required during pregnancy and lactation regarding maternal consumption of chemical agents, because the combination of intra-uterine growth and lactation allows maternal state to influence development during fetal life and infancy (Kiss *et al.,* 2012). In utero and postnatal exposure to chemicals from medicinal plants can modify brain growth of fetuses which may prompt the

development of many neural disorders (Vickers *et al.*, 2000). Many chemical substances transported across placenta and milk of rodents and humans (Taofeeq *et al*., 2005), imposes great risk to the offspring (Abebe *et al.,* 2022;). However, expectant and lactating mothers consume medicinal plants for it proposed nutritional values to better their health and that of the fetus and infants (Ukoh *et al.,* 2022). The intended effect is not always evident and the effects of such practices on the life of the young offspring are not clear (Van Der Kooi and Theobald, 2006;).

Jatropha tanjorensis is a perennial plant, it belongs to the family Euphorbiacere, conventionally known as "Hospital too far" (Oyewole *et al.*, 2012). The leaves are used locally to treat anaemia, diabetes and cardiovascular diseases, and it is anticipated from local consumers that it has blood replenishing properties (Orhue *et al.*, 2008) and recommended for use in pregnancy (Falodun *et al.,* 2013). In an earlier study we observed that consumption of the plant improves fertility potential in virgin female rats and gestational outcome (Ukoh *et al.,* 2022), relieved postpartum-like behaviours in dams (Atim *et al*., 2023), and augments neuroendocrine hormones release which aids maternal caregiving, pups survival and growth (Ukoh *et al.,* 2024).

Despite the multipurpose usage of the plant their effect on neural development and cognitive function in young offspring exposed in-utero and postnatal is yet to be known. Therefore, this study seeks to assess cognitive functions and hippocampal tissue in juvenile rats exposed to aqueous leave extract of *Jatropha tanjorensis* (ALEJT) to untangle the claims whether or not *Jatropha tanjorensis* will be beneficial to human health.

MATERIALS AND METHODS

Plant Collection Authentication and Extraction

Fresh mature leaves of *Jatropha tanjorensis* were acquired from the vegetal garden of University of Calabar, Nigeria. A Voucher Specimen Number (Herb/Bot/UCC/182) deposited in the University Herbarium. Plants preparation was by the method of Agarwal *et al.*, (2007). The leaves were carefully washed with fresh water and air-dried at room temperature for 2 days, before drying in an oven (40°C) for 24 hours. The leaves crisp was grounded into powdered form and conserved in moisture-free, airtight containers pending usage. The plant powdered form (100 g) was softened with water (1000 ml) in ratio of 1:10 and agitation was done occasionally for 48 hours before filtering into a clean glass jar. Mixtures filtration was done using filter cloth, Whatman filter paper of pore size 11µm, and then filtrates was evaporated to complete dryness using a thermostatically controlled water bath at 42 ^{0}C.

The ALEJT was prepared by dissolving in distilled water (vehicle) at 20 ml/kg (2 ml/100g) body weight (OECD, 2000). LD50 was by the method of Lorke, (1983). *Jatropha tanjorensis* showed no overt signs of distress or toxicity even at a dose of 5000mg/kg body weight. Therefore, in this study the extract dosage of 500mg/kg was considered safe for pregnant and postpartum rats.

Animals

Forty albino rats (30 virgin females and 10 males) weighing averagely 120 -150g were purchased from the animal farm, University Calabar, Nigeria. The animals were kept under standard laboratory conditions and housed singly in well ventilated plastic cages at room temperature and relative humidity with light and dark cycles (12hr/12hr). The animals were acclimatized for one week and fed with typical rat pellet (Pfizer feed PLC, Lagos, Nigeria) and water ad libitum. Ethical approval was obtained from the University of Calabar College Ethical Committee on the use of experimental animals with protocol number (093PHY3321).

Experimental design

After acclimatization, the rats were housed singly in plastic cages, except for mating. A male rat was caged with three female rats on varying days, each female rat was returned to their cages while the next female rat was introduced. The appearance of vaginal plug was deemed day 1 of gestation. The inseminated females were randomly assigned three groups. Group 1 (control) received 0.2mL of normal saline (vehicle) throughout gestation; Group II (in-utero) received 500mg/kg of ALEJT once daily throughout gestation; while Group III (in-utero + postnatal) received 500mg/kg of ALEJT once daily throughout gestation and at postnatal days 15-21 via oral routes. All pregnant rats were allowed to deliver and wean their litters. After weaning (PND 21), 10 litters from each dam's group were randomly selected. The juvenile rats were kept under standard laboratory conditions, each groups were housed in well ventilated wooden cages at room temperature and relative humidity with light and dark cycles (12hr/12hr). Acclimatization was for two weeks and fed with normal rat pellet and water ad libitum. At 5 weeks of age (PND 35) experimental assay for cognitive functions commenced, followed by histological examination of the hippocampus to ascertain the effect of exposure to ALEJT in-utero and at postnatal life. This spans from PND 35 to PND 54.

Cognitive assay

Cognitive ability and visuo-spatial memory and learning status were assessed using MWM Test (Bisong *et al.*, 2010), NORT (Ennaceur and Delacour, 1988) and T maze alternation test.

Morris water maze

The test apparatus was made of a circular swimming pool (180 cm in diameter and 60 cm high). The maze was placed in a room with frequent visual cues. The pool was filled with water and kept overnight to acclimatize to room temperature. The water was then

made opaque with milk. The pool was sectioned virtually into four equal quadrants, as Northwest, Northeast, Southwest and Southeast. The quadrants boundaries were marked on the edges of the pool using masking tape and labeled: North, South, East and West. A platform (12.5 cm in diameter and 38 cm high) which served as a refuge from the water was placed in one of the four maze quadrants (the target quadrant) and submerged 2.0 cm below the water surface.

The test took 8 days (PND 40-47):

Days 1-3: Acquisition days
Days 4-6: Reversal days
Days 7: Probe trial
Days 8: Visible-platform day

Acquisition and reversal training were done with the hidden platform (water is 0.5cm above platform).

During acquisition training, the platform was placed in the center of the Northeast quadrant. The starting points of the rat were predetermined using a Latin square design, to prevent the repetition of starting point sequences on different test days. Possible start positions were at the boundaries of the quadrants (West, North, East or South). For each trial, the rat was picked at the base of its tail and then placed into the water at the appropriate start point.

The rat was allowed to explore the pool and search for the hidden escape platform for 60 seconds. When the animal located the platform, the manual timer was stopped and the rat remains on the platform, if the rat did not locate the platform during the 60 second interval, the animal was led onto the platform using the plastic container. Once on the platform, the rat was allowed to view the extra-maze environment for 10 seconds and to associate the platform with escape before returning it to a holding cage with strips of paper towel in the bottom to allow the animal dry quickly to avoid hypothermia. This paper towel was replaced when it became wet. Each animal completed 4 trials per day over 3 days, for 12 trials of acquisition training, each trial from a different one of the 4 start locations.

Reversal training began on day 4. The hidden platform was moved to the opposite quadrant (Southwest quadrant), and rats were again assigned to appropriate start points. The same procedures as in acquisition training were carried out during reversal training. Each of the animals completed 4 trials per day for 3 days for a total of 12 trials of reversal training.

A probe trial was done on day 7 to assess visuo-spatial memory. In this trial, there was no escape platform in the pool. Each rat was placed in the pool from one of the four possible start positions and allowed to explore the pool for 60 seconds, and the time spent in each quadrant was recorded. At 60 second interval, the rat was scooped up using a container and placed in a holding cage with strips of paper towel in it bottom.

The visible platform task was conducted on day 8. The visible platform was placed in a new location within the Northwest quadrant of the pool. The same procedures as in acquisition and reversal training were repeated and rat completed 4 trials.

Behaviours scored: Swim latency (the time taken for the animal to locate the platform); Annulus acquisition crossing (the number of times the rat crossed the location of the platform during the acquisition training); Annulus reversal crossing (the number of times the rat crossed the location of the platform during the reversal training); Quadrant duration (time the animal spent in each quadrant).

Novel object recognition test

The NORT evaluates rodent's ability to recognize a familiar object over a variable length of time; this ability is called recognition memory (Atim *et al.,* 2023).

The test was done on PND 35-36. Before the test, all rats were allowed to familiarize with the apparatus for 5 minutes. After each 5 minutes trial, the rats were returned to their cages and the apparatus cleaned with 70 % ethyl alcohol and permitted to dry between trials.

Trial 1 (acquisition): On day 1, each rat was placed in the arena with two identical objects (N1 and N2) placed in diagonal corners opposite each other in the open field. Exploration time was 5 min.

Trial 2 (recognition): On day 2, one of the object (N1 or N2) was replaced with a novel object (O) and the experiment repeated for each rat for 5 minutes. The behavioral score of trial 1 and 2 were recorded using an electronic stop watch.

Behaviours scored include discrimination Index (time spent between the novel and familiar objects) and Index of habituation (comparing the total time spent in exploring the two objects during the familiarization phase and the test phase).

T-maze spontaneous alternation test

The T-maze is an elevated apparatus in the form of a 'T' placed horizontally. It was made of wood and consists of three arms, the starting arm is the base of the T-maze (50cm x 16cm) and two goal arms on either side (50cm x 10cm) with a wooden door that fit at it entrance. It gave the rats two environment to explore. The enclosed T-maze had a wall height of 30cm.

On PND 38, each rat was placed on the maze and allowed to explore it for 5 min. This maze was used for 2-trial and the maze was cleaned using 70% ethanol

and allowed to dry between each trial. Trial 1 commenced at 10 minutes, the left and right arm doors of the maze were opened and the animals were placed at the base of the T Maze, stop watch was used to record the exploration time of the goal arms. When all four paws of the rats entered one arm, the arm door was closed for 2 minutes and arm entry recorded as R (right arm) or L (left arm) entry. Trial 2 was done after 15 minutes as Trial 1.

Spontaneous alternation behavior (SAB) describes the natural tendency of rodents to alternate which arms of a maze they choose to explore. SAB was recorded if the rat chose the alternate arm in Trial 2. Rodents prefer to visit the less recently visited arm, meaning that it will need to recall which was the last arm visited. The percentage alternation was as reported by Sivakumar *et al.,* (2017).

Percentage alternation:

$$\frac{\text{The number of correct choices}}{\text{Total sets performed}} \times 100$$

Tissue processing

Immediately after neurobehavioral studies, the rats were sacrificed after anaesthetizing with 60 mg kg^{-1} of ketamine-hydrochloride (#50155, Rotex Medica, Trittau, Germany). Intracardial perfusion with phosphate-buffered saline (PBS, 2M, pH 6.4), was carried out on the rats using a cannula and then perfusion-fixed using 10% buffered formalin. On complete perfusion, the skull was opened and the brain removed and post-fixed in 10% buffered formalin for 48 hours. The whole brain was further routinely processed for haematoxylin and eosin (H&E) histological studies (Neurotech, 2012). Sections were then observed under light microscope and photomicrographs were obtained using the microscope camera linked to a computer.

Histological studies

The hippocampus was isolated from rats, stored separately in 10 % buffered formalin and further histologically processed for H&E staining. Section containing the hippocampus was float mounted on microscope slides, and then submerged in varying grades of alcohol (2 min each): 100 %, 95 %, and 70 % ethanol. Sections were rinsed in distilled water to remove excess ethanol and placed in H&E staining solution in distilled water for 3 min. Sections were rinsed in distilled water to remove excess stain and immersed in a 0.8 % acetic acid solution in distilled water until fiber tracks became unstained (about 3-5 min). They were then placed in 70 %, 95 % and 100 % ethanol for 2 min each. Sections were placed in clearance solution for a minimum of 15 min before they were cover slipped with mounting medium. Photographic images of the different cell layers were captured using Olympus camera (E-330) and Olympus microscope (CX-61) with a motorized stage. Photomicrographs were taken at magnifications of ×400 using a light microscope (Leica DM 750, Switzerland).

Statistical Analysis

All experimental data were expressed as mean ± standard error of mean (SEM), with the aid of SPSS version 24.0 and GraphPad Prism version 7.0 statistical software. Significant differences were analyzed using one-way ANOVA with Turkey multiple comparison test and Pearson Chi-Square. The results obtained were considered as statistically significant if $p < 0.05$.

RESULTS

Effect of gestational and lactational exposition to aqueous leaf extract of *Jatropha tanjorensis* on cognitive function in rats using novel object recognition task test.

Index of habituation

The index of habituation for short term memory (STM) for control litters, litters exposed to ALEJT in-utero and litters exposed to the extract in-utero and at postnatal days 15-21 are: -10.63±8.09, 18.17±5.70 and 17.50±7.05 respectively. The index of habituation for STM of litters exposed to the ALEJT in-utero (0.01) and in-utero + postnatal ($p< 0.05$) was significantly higher compared to control. This is shown in Fig. 1A.

The index of habituation for long term memory (LTM) for control, litters exposed in-utero and in-utero + postnatal to the ALEJT are: -5.27±3.50, -2.41±9.30 and 17.7±6.61 respectively. The index of habituation for LTM increased significantly in the in-utero + postnatal exposed group ($p< 0.05$) when compared to control. There was no significant different in the exposed in-utero compared to control. This is shown in Fig. 1B.

Index of discrimination

The index of discrimination for STM for control litters, in-utero and in-utero + postnatal ALEJT exposed groups: 0.43±0.18, 0.39±0.12 and 0.24±0.07 respectively. Fig. 2A shows that these did not differ significantly in the exposed groups compared to the control.

The index of discrimination for LTM for control litters, litters exposed to the ALEJT in-utero and in-utero + postnatal are: 0.15±0.09, -0.27±0.06 and -0.13±0.22 respectively. The index of discrimination was decreased significantly in the in-utero exposed group when compared to control ($p<0.05$). There was not significant different in the in-utero + postnatal exposed group compared to control. This is shown in Fig. 2B.

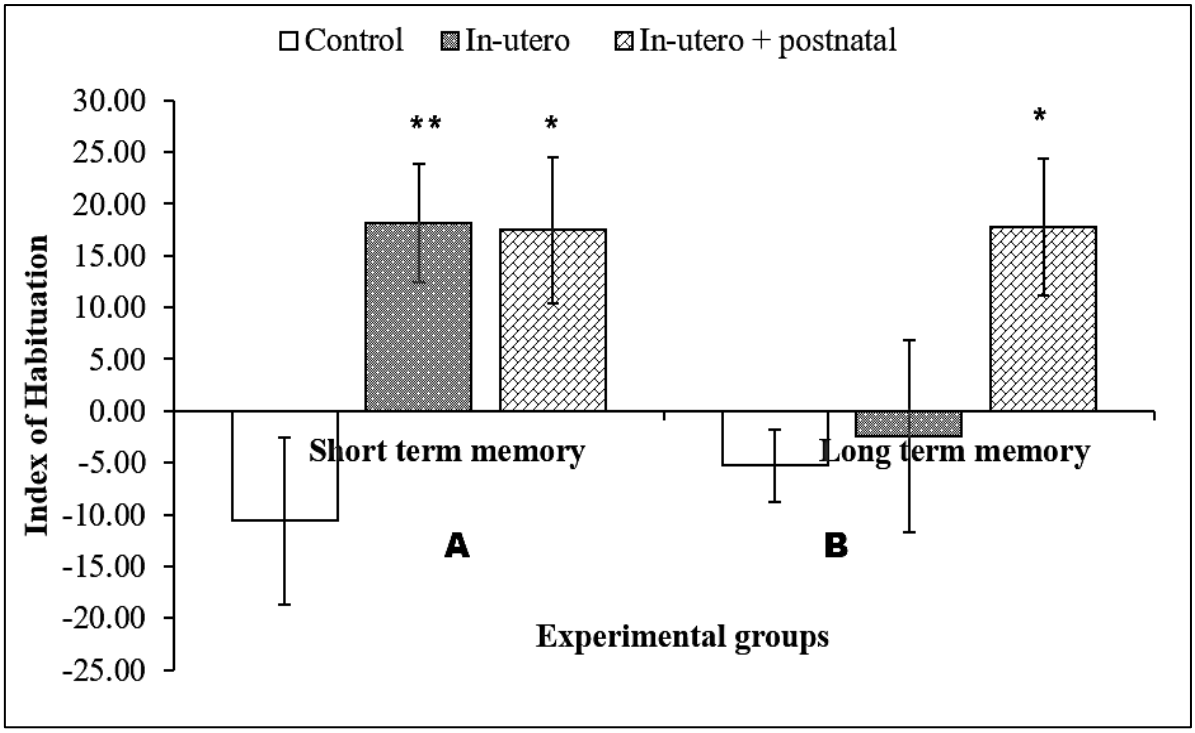

Fig. 1: Comparison of index of habituation for NORT in the rats' offspring.
n=10; *P<0.05 compared to control, **p< 0.01compared to control.

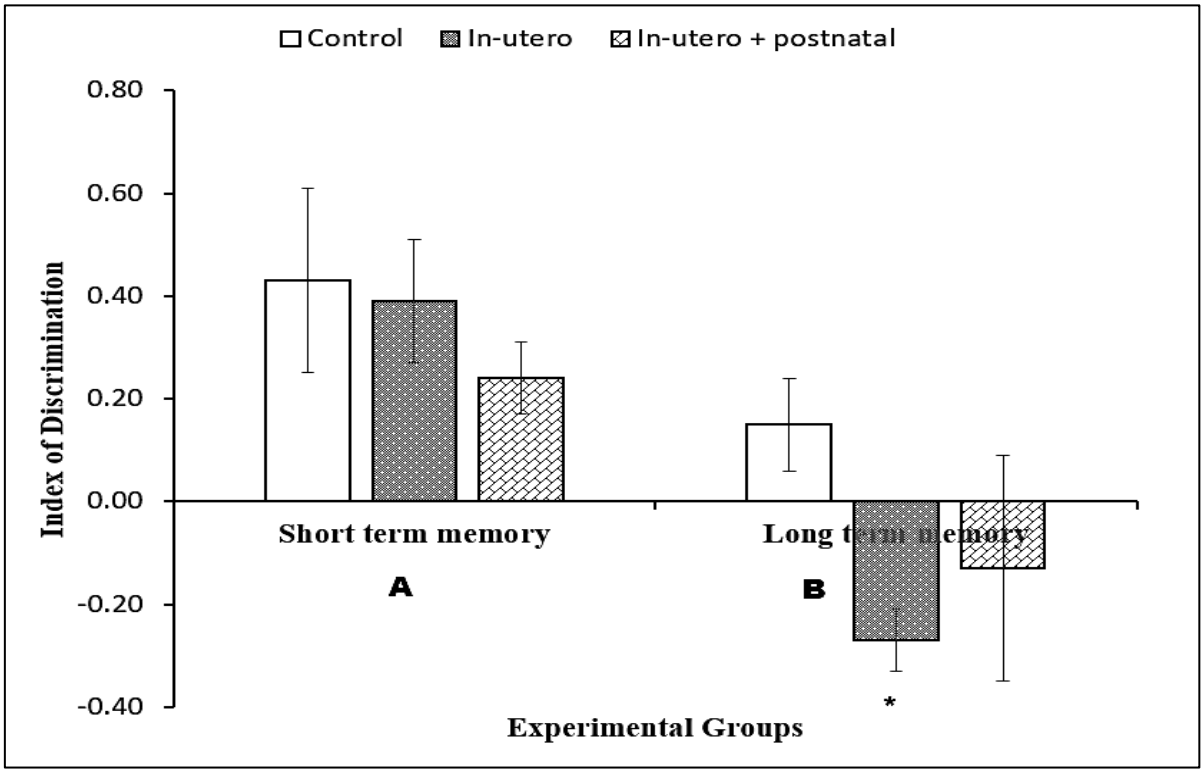

Fig. 2: Comparison of index of discrimination for NORT in the rats' offspring.
n=10; *P<0.05 compared to control

Effect of gestational and lactational exposition to aqueous leaf extract of *Jatropha tanjorensis* on cognitive function in rats using Morris water maze test.

Acquisition training

The swim latency for acquisition training day one for control litters, litters exposed to ALEJT in-utero and those exposed in-utero and at PND 15-21 are:

43.81±4.16, 45.86±4.86 and 41.47±4.96 respectively as shown in Fig. 3.

The swim latency for acquisition training day two for control litters, litters exposed to ALEJT in-utero and those exposed in-utero and at PND 15-21 are: 25.33±5.59, 27.90±3.41 and 22.11±4.01 respectively as shown in Fig. 3.

The swim latency for acquisition training day three for control litters, litters exposed to ALEJT in-utero and those exposed in-utero and at PND 15-21 are: 20.61±1.64, 16.47±2.13 and 12.47±2.03 respectively. The swim latencies for day 3 was significantly shorter in the group exposed in-utero and at postnatal ($p< 0.01$) compared to the control as shown in Fig. 3.

Reversal training

The swim latency for reversal training day one for control litters, in-utero and in-utero + postnatal *Jatropha* exposed groups are: 33.43±2.78, 37.79±4.81 and 19.39±2.91 respectively. The swim latency of in-utero + postnatal exposed group was significantly shorter ($p< 0.05$) compared to the control and significantly shorter ($p< 0.01$) compared to in-utero exposed group as shown in Fig. 4.

The swim latency for reversal training day two for control litters, litters exposed to ALEJT in-utero and those exposed in-utero and at PND 15-21 are: 15.93±3.51, 22.00±3.83 and 9.92±1.20 respectively. The swim latency of in-utero + postnatal exposed group was significantly shorter ($p< 0.05$) compared to group exposed in-utero as shown in Fig. 4.

The swim latency for reversal training day three for control litters, litters exposed to ALEJT in-utero and those exposed in-utero and at PND 15-21 are: 16.32±0.96, 9.89±1.84, and 9.33±1.28. The swim latencies of the exposed groups were significantly shorter ($p<0.01$) compared to control. This is shown in Fig. 4.

Quadrant duration

The quadrant duration for the North West (NW) quadrant for control litters, litters exposed to ALEJT in-utero and those exposed in-utero and at postnatal days 15-21 are: 16.18±1.19, 14.44±1.02 and 18.06±1.04 respectively. There was no significant difference in quadrant duration as shown in Fig. 5.

The quadrant duration for the South East (SE) quadrant for control litters, litters exposed to ALEJT in-utero and those exposed in-utero and at PND 15-21 are: 10.53±0.89, 13.01±0.78 and 11.57±0.91 respectively. The quadrant duration for the NW in Group 3 increased significantly ($p< 0.05$) compared to Group 2 as shown in Fig. 5.

The quadrant duration for the North East (NE) quadrant for control litters, litters exposed to ALEJT in-utero and those exposed in-utero and at PND 15-21 are: 13.98±1.02, 11.99±1.09 and 11.29±1.11 respectively. There was no significant difference in quadrant duration as shown in Fig. 5.

The quadrant duration for the South West (SW) quadrant for control litters, litters exposed to ALEJT in-utero and those exposed in-utero and at PND 15-21 are: 18.98±0.68, 20.55±0.56 and 18.73±0.74 respectively. The quadrant duration in the South West quadrant was significantly higher the group exposed in-utero and at postnatal ($p< 0.05$) compared to group exposed in-utero as shown in Fig. 5.

Annulus acquisition

The annulus acquisition for control litters, litters exposed to ALEJT in-utero and those exposed in-utero and at PND 15-21 are: 1.63±0.51, 1.88±0.35 and 1.75±0.25 respectively. This did not differ significantly between groups.

Annulus reversal

The annulus reversal for control litters, litters exposed to ALEJT in-utero and those exposed in-utero and at PND 15-21 are: 3.13±0.48, 3.38±0.32 and 3.88±0.31respectively. This did not differ significantly between groups.

Visible platform

The swim latency during the visible platform for control litters, litters exposed to ALEJT in-utero and those exposed in-utero and at PND 15-21 are: 17.81±2.61, 15.14±2.46 and 8.95±0.98 respectively. The swim latency of the group exposed in-utero and at postnatal was significantly lesser ($P<0.01$) compared to control as shown in Fig. 6.

Effect of gestational and lactational exposition to aqueous leaf extract of *Jatropha tanjorensis* on cognitive function in rats using T-maze spontaneous alternation test.

Chi square statistics were used to examine association between categorical variables. There was no significant relationship at 5% significance level between experimental groups and spontaneous alternation behaviour ($x^2= 4.267$, df=2, P=.118).

Figure 7 shows comparison of percentage alternations scored in the exposed groups and control. This did not differ significantly between groups.

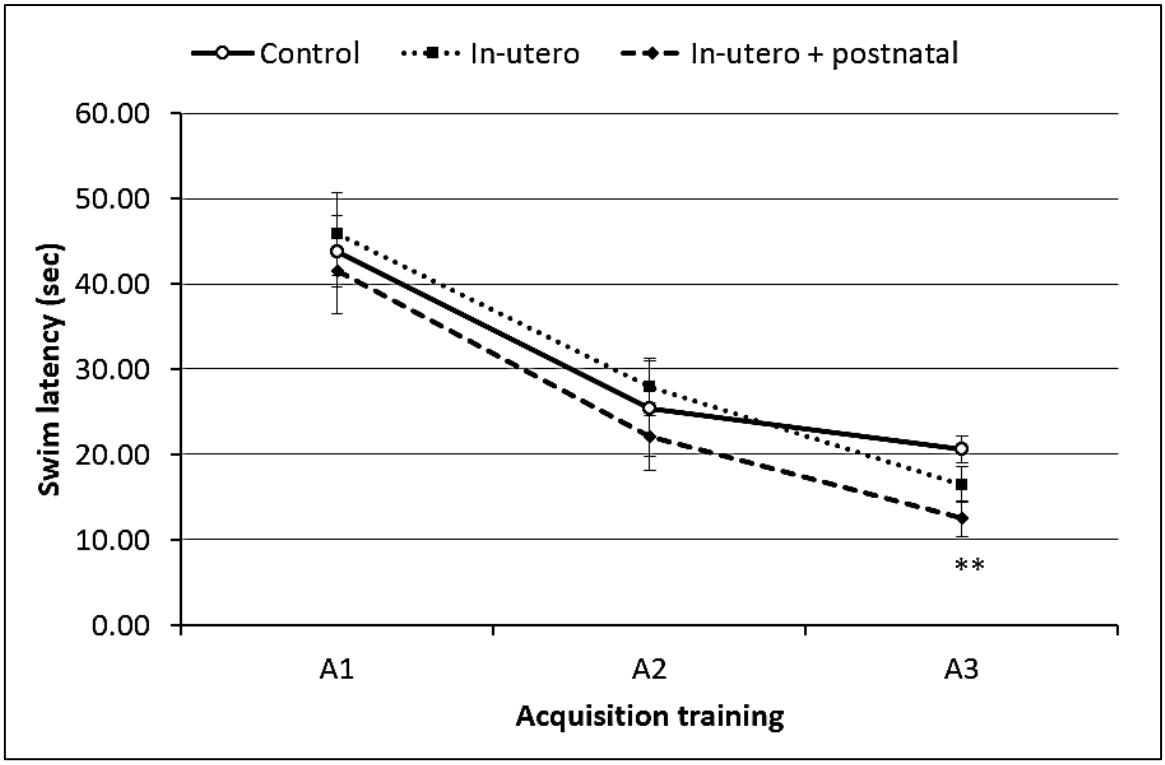

Fig. 3: Learning curves showing comparison of swim latencies during three days of acquisition training (A1-A3) in the MWM test of rats' offspring
n=10; **P<0.01 compared to control.

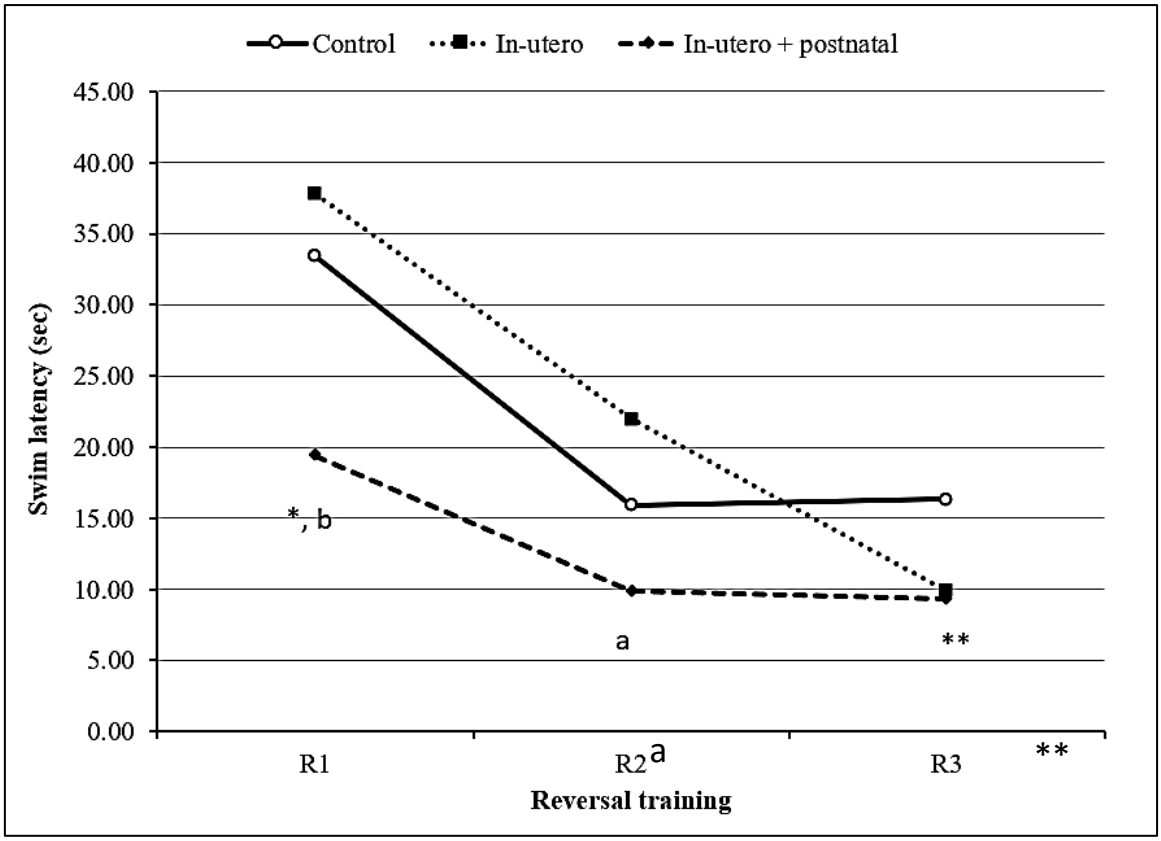

Fig. 4: Comparison of swim latencies during three days reversal training (R1-R3) in the MWM test of rats' offspring
n=10; *P<0.05; ** P<0.01 compared to control

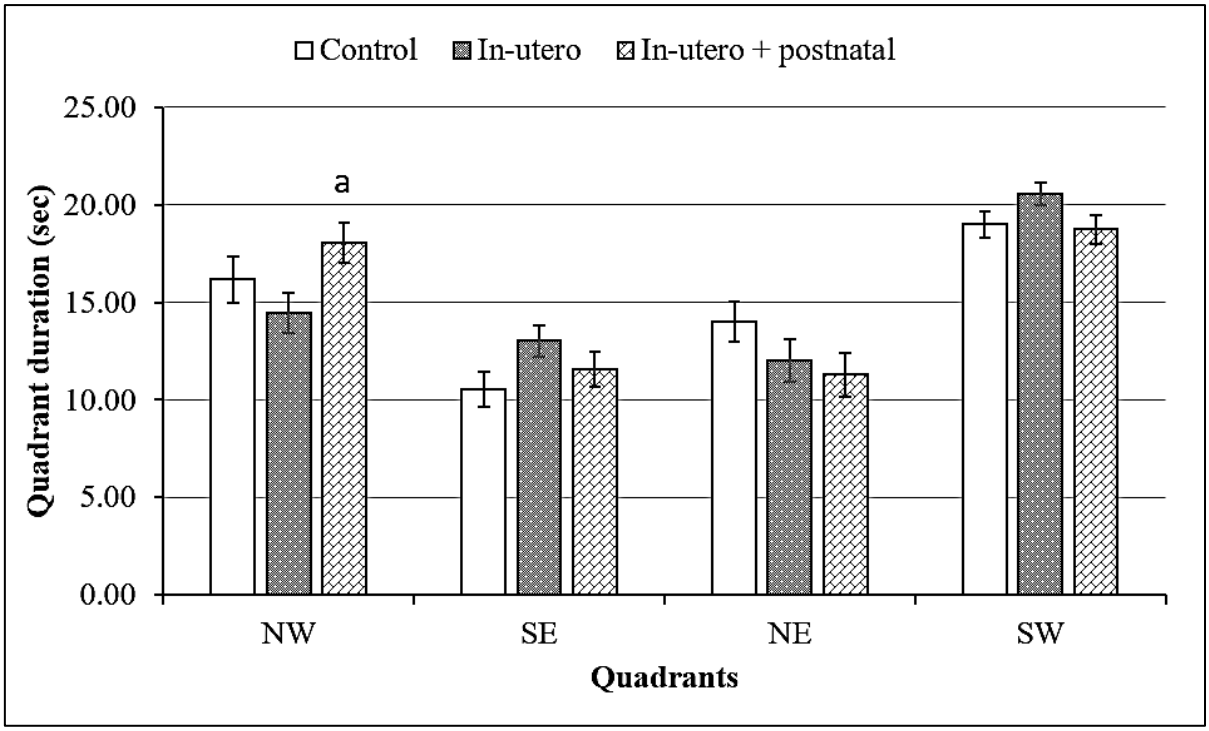

Fig. 5: Comparisons of quadrant duration in the MWM test of rats' offspring. n=10; a= p<0.05 compared to in-utero group

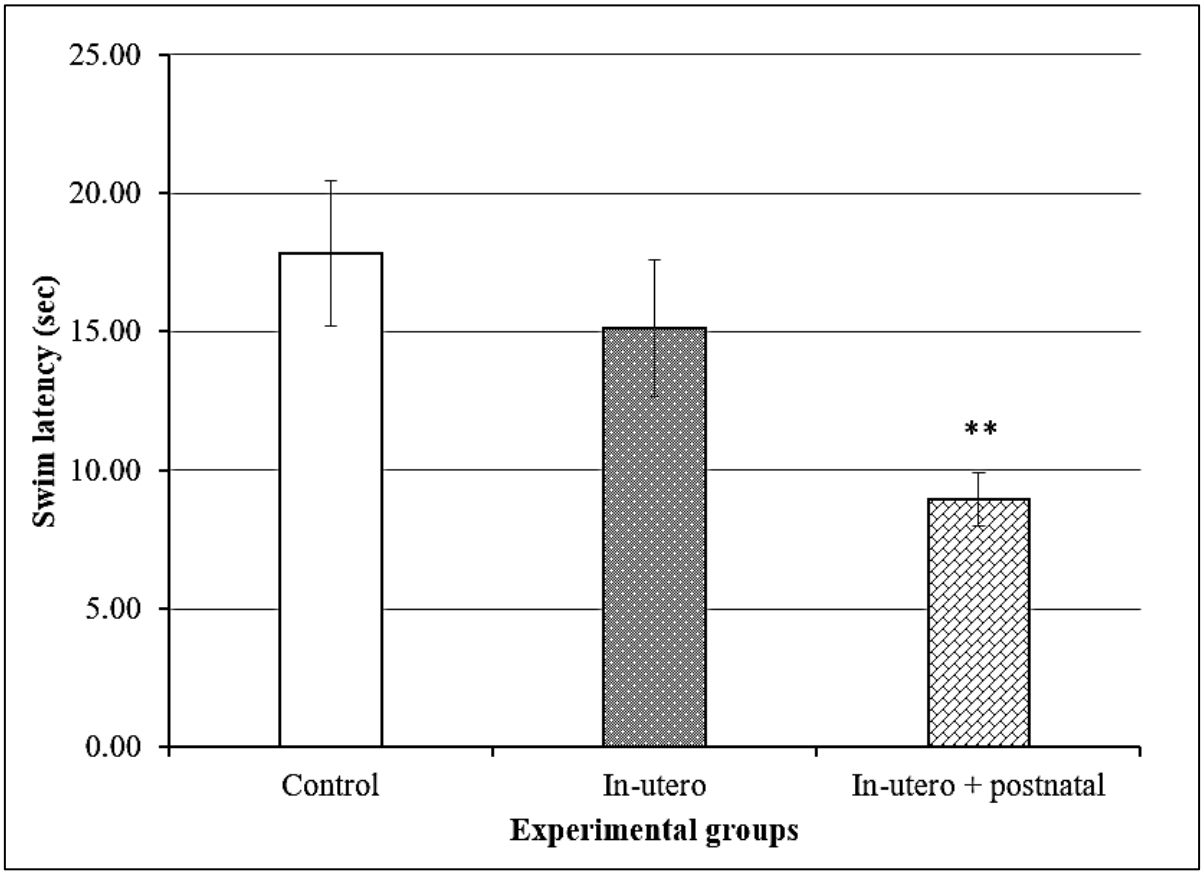

Fig. 6: Comparisons of swim latency during the visible platform in the Morris water maze test of rats' offspring. n=10. **p<0.01 compare to control

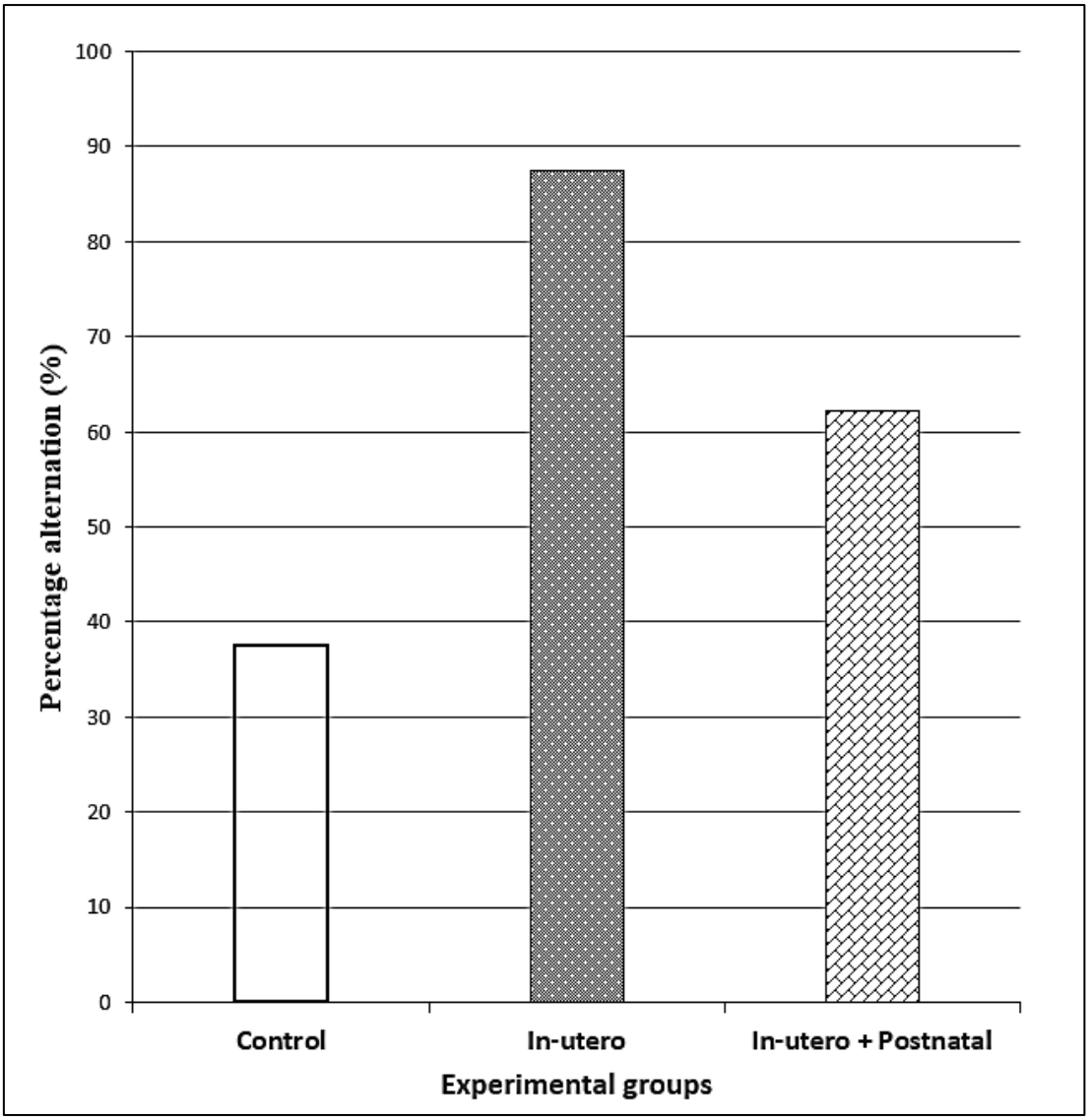

Fig. 7: Comparison of percentage alternations scored in the T-maze of rats' offspring. n=10.

Chi square statistics were used to examine association between categorical variables. There was no significant relationship at 5% significance level between experimental groups and spontaneous alternation behaviour (x^2= 4.267, df=2, p=.118)

Effect of gestational and lactational exposition to aqueous leaf extract of *Jatropha tanjorensis* on neurodevelopment in rats histological examination.

Photomicrographs of hippocampus

The photomicrograph of hippocampus at magnification of X400 for the control litters (2A) showed evenly distributed molecular cell layer (MCL). The pyramidal cell layer (PYL) consists of pyramidal cells with triangular shape bodies having prominent round to oval nuclei and pigments within their cytoplasm. Scattered around the pyramidal cells were microglia and astrocytes. The polymorphic layer (POL) consists of abundant neuropil comprising of neuronal cell processes and neuroglia.

The photomicrograph of hippocampus of the group exposed in-utero (2B) showed evenly distributed MCL. The PYL showed pyramidal cells with reduced number of neuronal cells, pyramidal cells hypertrophy, with moderate amount of cytoplasm. Scattered around the pyramidal cells were microglia and reactive astrocytes. The POL showed abundant neuropil comprising of neuronal cell processes and neuroglia.

For group exposed in-utero and in postnatal period (2C) the photomicrograph of hippocampus showed distributed MCL. The PYL showed loosely packed pyramidal cells with triangular shape bodies having prominent round to oval nuclei and pigments within their cytoplasm. Scattered around the pyramidal cells were microglia and reactive astrocytes. The POL showed abundant neuropil comprising of neuronal cell processes and neuroglia.

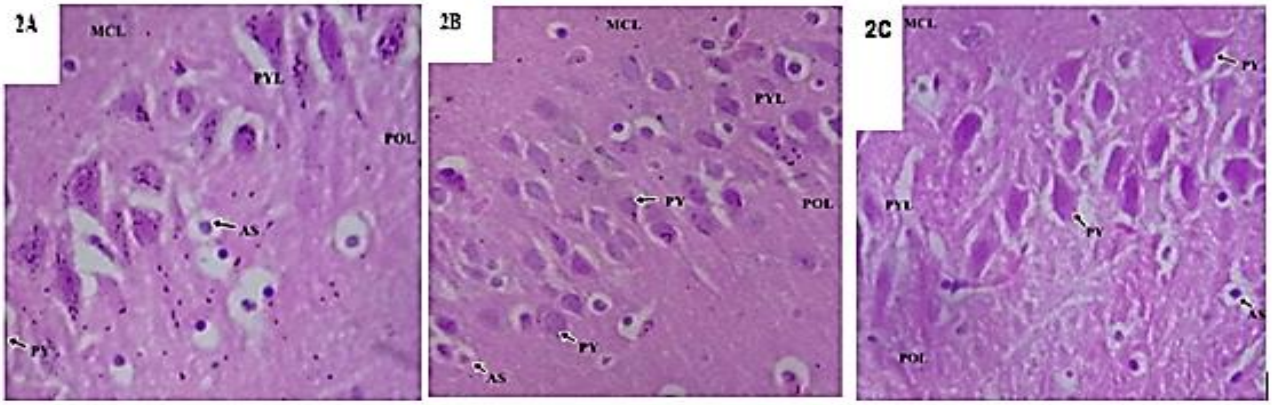

Plate 1: Photomicrograph of hippocampus between the control and *Jatropha* exposed groups Magnification: x400

KEY:

2A: Control group, showing evenly distributed MCL (molecular cell layer), PYL (pyramidal cell layer) having pigments within their cytoplasm, AS (astrocyte) and macroglia scattered around the pyramidal cells (PY) and POL (polymorphic layer) having abundant neuropil comprising of neuronal cell processes and neuroglia

2B: Group exposed in-utero, showing evenly distributed MCL, PYL with reduced number of neuronal cells and cells hypertrophy with moderate amount of cytoplasm, scattered microglia and reactive astrocytes (AS) around the pyramidal cells (PY) and POL having abundant neuropil comprising of neuronal cell processes and neuroglia.

2C: Group exposed in-utero + postnatal, showing distributed MCL, loosely packed PYL with triangular shape bodies having prominent round to oval nuclei and pigments within their cytoplasm, scattered microglia and reactive astrocytes around the pyramidal cells (PY) and POL having abundant neuropil comprising of neuronal cell processes and neuroglia

4.0 DISCUSSION

Many studies have already evaluated the effectiveness of *Jatropha tanjorensis*, but nothing has been done so far to investigate it effects on the life of exposed offspring. Hence, this study was set to assess cognitive function and hippocampus morphology in juvenile rats exposed to ALEJT in-utero and postnatal life. The exposed groups were tested for NORT, MWM and SAB.

NORT is used to assess recognition memory in rat (Sik *et al.*, 2003). Index of habituation was determined by comparing total time spent in exploring two objects in familiarization phase and test phase. The exposed groups spent more time in familiarization phase exploring both objects against test phase for STM, while LTM in Group 3 spent more time during familiarization phase exploring both objects against test phase. Broadbent *et al.,* (2010) reported that the less time animals spent exploring the objects during familiarization phase, the stronger was the novel object preference during test phase. Index of discrimination is based on the belief that when animals are exposed to a familiar and a novel object, they approach recurrently and spend more time exploring the novel than the familiar one because presentation of the familiar object exists in the animals' memory (Ennaceur 2010). This result vary between +1 and -1, where a positive score indicates more time spent with the novel object, a negative score indicates more time spent with the familiar object, and a zero score indicates a null preference (Aggleton *et al*., 2010; Burke *et al*., 2010). STM did not differ, while for LTM, the negative score of the exposed groups meant less time was spent exploring the novel object. This indicated that ALEJT exposure reduced ability of rats to recognize novel objects, signifying poor learning and impaired memory.

MWM test is used to assess cognitive ability of pharmacological treatments and the effect of the maze parameter on differences in spatial learning (Chapillon and Debouzie, 2000). The hidden platform version (acquisition and reversal training) is a test of visio-spatial learning and memory (McDonald and White, 1994). Group 3 showed shorter swim latency from day 3 of acquisition till the last day of reversal training but the learning curve was not better, while Group 2 did not show a stronger relationship between the number of trials and the swim latency. The shorter the swim latencies, the better the learning processes. Also the steeper the gradient of swim latency the better the learning curve, which signifies learning (Aduema and Wariso, 2017). Hence, exposure to ALEJT in-utero and/or at postnatal life displayed impaired learning ability. During probe trial (visio-spatial memory assessment), it is expected that rat which have a good memory of the spatial location of the hidden platform would spend more time exploring the quadrant which had the platform during the reversal training, in this case, the SW quadrant. Group 3 spent more time in the northwest quadrant signifying poor memory. The visible platform is used to assess cue learning and visual integrity of the animals (Aduema and Wariso, 2017). During this phase, Group 3 showed

shorter swim latency which indicates improved cue learning.

SAB is a typical manifestation of short-term spatial working memory. Working memory keeps incoming information for a short time while deciding what to do (Ganong, 2005). In the present study, the alternation scored did not differ significantly.

H&E staining contrasts the different cellular elements in tissues (Wippold and Perry, 2006) and can be used to intuitively evaluate the degree to which toxic substances alter tissue morphology (Xie *et al.,* 2023). H&E staining was used to observe the changes in hippocampal morphology after ALEJT exposure. From the results, exposure to ALEJT altered the pyramidal neuronal cells of the hippocampus. Lapp *et al.,* (2023) reported that in-utero exposure is potential contributors to susceptibility for neurodevelopmental disorders. Ishola and Adeniyi, (2014) observed retarded hippocampal development following prenatal exposure to ethanolic leaves extract of Datura metel in wistar rats. Hippocampal pyramidal neurons in rat are generated prenatally (Semple *et al.,* 2013), and are the principal excitatory cell types of the hippocampus which processes input from other brain areas (Baker *et al.,* 2018) and transmits all hippocampal output to various targets throughout the brain (Graves *et al.,* 2012), depicting their roles in advanced cognitive functions (Nelson, 2009). Graves *et al.,* (2012) stated that pyramidal neurons can be modulated in several ways to alter information processing and cognitive ability. H&E staining also showed the presence of reactive astrocytes in the pyramidal cells of both exposed groups. In the rat, astrocytes maturation occur in the first few postnatal weeks (Semple *et al.,* 2013), and astrocytes exhibiting reactive phenotypes are due to their distinct changes in response to pathological stimuli, which results in the loss of their usual astrocyte functions (Yang *et al.,* 2024).

Xie *et al.,* (2023) reported that changes in cognitive behaviors may be accompanied by changes in tissue morphology. As observed in the cognitive paradigms in this study, neuronal changes and the presence of reactive astrocytes in pyramidal cells may be responsible for cognitive deficits in the offspring. NORT is used to test the effects of various treatments in hippocampus damage (Goulart *et al.,* 2010) and Elston, (2003) reported that pyramidal cells play a critical role in complex object recognition. The hidden platform version of the MWM is sensitive to hippocampus injury (Aduema and Wariso, 2017). T-Maze spontaneous alternation test is also used for detecting hippocampal dysfunction (Deacon, and Rawlins, 2006).

However, this study was limited because of lack of funding; we couldn't access the active ingredient in ALEJT that crosses the placenta barrier to induce neurotoxicity & impair cognitive functions. More animal studies, in-utero and in postnatal life are required to verify the link between ALEJT early exposure to immature brain and the possible neurotoxicity.

CONCLUSION

Early environmental exposures in-utero and postnatal life is potential contributors to vulnerability for neurodevelopmental disorders. This study indicates cognitive deficit after exposure to ALEJT in-utero and/or postnatal life. Morphological study of the hippocampus further shade more light on the toxicity of the extract. If these results are applicable to human, consumption of ALEJT could be harmful in reducing cognitive function in human.

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